

Determination of Di-2-ethylhexyl Phthalate Levels in Human Blood Plasma and Cryoprecipitates

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While investigating the possible occurrence of neutral plasmalogens in human blood, we isolated a substance which had the same chromatographic characteristics on thin-layer chromatoplates as those of alkyl diglycerides. This substance was identified (1) as di-2-ethylhexyl phthalate (DEHP), and we were able to demonstrate (2) that this plastifier originated from the plastic transfusion packs which had contained the blood and that its concentration increased with the time of storage up to a level of about 11.5 mg/100 ml of plasma after 21 days. These observations were confirmed by Jaeger and Rubin (3), who have also shown that DEHP accumulates in liver cells during the perfusion of rat liver. These authors also found that liver, spleen, lung, and abdominal fat in two patients who had received blood transfusions contained significant quantities of DEHP.

In the present report, we describe a direct method of determination of DEHP by gas-liquid chromatography. With this method, we have been able to demonstrate that the leaching out of DEHP from plastic transfusion packs was correlated with the plasma concentration of triglycerides. Also the DEHP levels in cryoprecipitates, which are

prepared from blood contained in plastic transfusion packs, have been measured.

Methods

Blood Samples and Cryoprecipitates

The blood samples were taken with evacuated glass tubes from transfusion blood bags which had been stored for 24 days in a local blood bank. The cryoprecipitates were stored frozen in plastic transfusion packs and had been prepared by the standard method used by the Canadian Red Cross. The cryoprecipitates were thawed at room temperature and their liquid contents were drained by gravity to be extracted for lipid analysis.

Plasma Lipid Determination

The plasma concentration of triglycerides was measured with the semi-automated method of Kraml and Cosyns (4) and that of total cholesterol was determined by the method of Block et al. (5) with an auto-analyzer.

Determination of DEHP Concentration

To an aliquot of plasma (1 ml), methanol (5 ml) was added, followed by addition of dipentyl phthalate or dipropyl phthalate as an internal standard. For optimum accuracy,

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the concentration of the internal standard (100–400 μg) is adjusted to be in the same range as that of DEHP. Chloroform (5 ml) was added and the mixture thoroughly shaken. Then chloroform (2.5 ml) was added and the mixture was shaken again. After standing at room temperature for 30 min, 2.5 ml of NaCl solution (0.75%) was added, and the two phases thus formed were slowly inverted. After separation of the phases overnight at 4°C, the upper aqueous phase and the proteins were removed by suction, and the chloroform phase was evaporated under a stream of nitrogen. The lipid extract thus obtained was dissolved in chloroform (100 μl) and an aliquot (2 μl) was injected in a gas chromatograph equipped with a hydrogen flame detector and an electronic integrator. For this analysis two stationary phases were alternatively used: a polar phase of diethylene glycol succinate packed in a 4-ft column which was operated isothermally at 195°C or a nonpolar phase of dimethyl silicone gum packed in a 2-ft column which was operated at the initial temperature of 175°C with an increment of 2°C/min. In both cases, the separation was satisfactory, and no lipid fraction was found to interfere with the peaks of phthalates. The concentration of DEHP was then calculated from the ratio of the peak areas of DEHP and from the concentration of the internal standard.

Results and Discussion

We have determined the DEHP contents and the cholesterol and triglyceride concentrations in 17 plasma samples which had been stored for 24 days in plastic transfusion packs, and the results are summarized in Table 1. The levels of DEHP found in the present study are significantly higher than those reported previously (2). This difference is probably due to the fact that in the previous study we had used preparative thin-layer chromatography to enrich the lipid extract in its DEHP content, a step which causes losses in DEHP. There is a strong positive correlation between the DEHP levels and the triglyceride concentra-

Table 1. Lipid concentrations and DEHP levels in plasma obtained from blood stored in plastic transfusion packs.

	\bar{X}	$\pm\text{S.D.}$	Range
Triglycerides, mg/100 ml plasma ^a	198	143	32 – 550
Total cholesterol, mg/100 ml plasma ^a	160	18	136 – 192
DEHP, mg/100 ml plasma ^a	14.5	2.9	10.6 – 20.9

^a($n = 17$).

tions ($r = 0.649$, $p < 0.01$), whereas there is no correlation between DEHP and total cholesterol. Therefore it appears that the leaching out of DEHP is directly related to the triglyceride content of plasma.

With the same method, we measured the DEHP content in bags of cryoprecipitates which are fractions rich in Factor VIII or antihemophilic factor and which are obtained from blood stored in plastic transfusion packs. The analyses were done on four cryoprecipitate packs, which contained respectively, 0.8, 1.2, 1.5, and 1.9 mg of DEHP. Although the DEHP content of cryoprecipitate bags is much lower than that of blood bags, this small concentration of DEHP may represent a serious problem for the hemophiliacs. Indeed many hemophiliacs, who are given a prophylactic or maintenance treatment with cryoprecipitates, receive about 400 bags of cryoprecipitates in a 1-year period. Therefore over the years it will be grams of DEHP that these patients will have received and probably accumulated in various organs. As Jaeger and Rubin have already demonstrated (3), DEHP does not appear to be metabolized and can accumulate in tissues like spleen, liver, lungs, and abdominal fat. Further, it is not certain that all aspects of the possible toxicity of DEHP have been investigated. Indeed, Rogers and Dunn (6) have reported that several cases of neonatal intestinal perforations which followed exchange transfusions may be attributable to substances such as plastifiers which leached out from the perfusion equipment.

All this evidence certainly warrants further studies on the presence and the effect of DEHP in human subjects and especially in haemophiliacs as well as in cases of multiple perfusions.

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